ASSESSMENT OF DEGRADATION FOR POROUS PCL-PLLA SEMI-IPN SHAPE MEMORY POLYMER (SMP) IMPLANTS FOR CRANIAL BONE DEFECT REPAIR

An Undergraduate Research Scholars Thesis

by

ABIGAIL A. ROTH

Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. Melissa A. Grunlan

May 2018

Major: Biomedical Engineering

TABLE OF CONTENTS

ABSTRACT

Assessment of Degradation for Porous PCL-PLLA Semi-IPN Shape Memory Polymer (SMP) Implants for Cranial Bone Defect Repair

Abigail A. Roth
Department of Biomedical Engineering
Texas A&M University

Research Advisor: Dr. Melissa A. Grunlan
Dept. of Biomedical Engineering, Dept. of Materials Science, Dept. of Chemistry
Texas A&M University

Within cranial bone defect treatments, autografts remain the current gold standard for best healing outcomes. However, if the defect is of a unique shape, this process proves difficult and often requires additional surgeries. The work herein focuses on a regenerative approach utilizing a shape memory polymer (SMP) scaffold that can "self-fit" into a defect while maintaining important properties for healing (e.g. osteoconductivity, robustness, degradability).

Poly(ε-caprolactone) (PCL) is an extensively studied SMP but, alone, is limited in bone repair due to its relatively low modulus and slow degradation rate for adequate bone healing.[1-3] To improve these properties, our group reported SMPs comprised of a semi-interpenetrating network (semi-IPN) of cross-linked PCL diacrylate (PCL-DA) and poly(L-lactic acid) (PLLA), which have shown great potential.[4, 5] Here, we investigated the degradation behavior of porous PCL-PLLA semi-IPN SMP implants *in vitro* under both accelerated conditions and non-accelerated conditions towards ultimately predicting *in vivo* performance. Rapid degradation with greater PLLA wt% content was observed, along with mass losses up to ~9% at 5 months real-time degradation. Additionally, degradation was unaffected by the compressed implant "fitting" process, yet slightly accelerated by the application of a bioactive surface coating.

ACKNOWLEDGEMENTS

This work was supported by NIH NIDCR 1R01DE025886. I personally would like to thank both Dr. Lindsay Woodard and Dr. Melissa A. Grunlan for continuous and unending guidance and counsel. I would also like to thank the LAUNCH office and UGS program for allowing me this opportunity to display my research in an official setting as an undergraduate student.

SECTION I

INTRODUCTION

Biodegradable, porous SMP scaffolds are a promising solution to heal bone defects as they would be able to (1) facilitate neotissue ingrowth and nutrient and waste transport, (2) maximize interfacial contact with the surrounding native bone and (3) degrade upon tissue regeneration. Due to the shape memory nature, such scaffolds based on cross-linked poly(ε-caprolactone) diacrylate (PCL-DA) have been found to become malleable and amenable to "press-fitting" into model cranial bone defects when slightly heated ($T > T_{trans}$; T_{trans} = ~55 °C).[6] Upon subsequent cooling ($T < T_{trans}$; i.e. body temperature), the scaffolds maintain their new, rigid shape within the defect. This is enabled by the melting and recrystallization of "switching segments" (i.e. semi-crystalline lamellae) as well as "netpoints" (i.e. chemical cross-links) which define the original shape.[7] The "fitting" behavior is critical to achieving high contact with adjacent bone tissue. Such contact is required for osseointegration with the surrounding tissue and is a feature lacking in many autografting procedures.[8] Moreover, unlike common injectable fillers that cure *in situ*, the porous SMP scaffolds' properties (e.g. modulus, porosity, etc.) are conveniently established *a priori*.

Our group has previously reported extensively on the potential of a porous, SMP scaffold based on PCL-DA for cranial bone defect repair.[5] High porosity was observed in addition to excellent shape memory behavior. Additionally, a developed polydopamine coating was applied to scaffold surfaces and found to increase hydrophilicity, impart bioactivity and enable osteoinductivity, all towards the potential to bond well to adjoining bone.[6] Despite favorable scaffold properties, it was determined that an increase in scaffold rigidity, in addition to enhanced, controlled rates of scaffold degradation would ultimately improve healing outcomes.

In addition to poly(ε-caprolactone) (PCL), and poly(L-lactic acid) (PLLA) has been extensively studied for biomedical applications.[9] PLLA exhibits a high modulus and has been often copolymerized or physically blended with PCL to improve the mechanical properties of PCL. Some approaches have also yielded faster degradation rates versus PCL alone.[1, 2, 10] Still, such blends' and copolymers' degradation rates are anticipated to be still too slow for success in bone regeneration.

More recently, our group reported the properties of semi-interpenetrating networks (semi-IPNs) comprised of cross-linked PCL-DA and thermoplastic PLLA.[4, 11] Initially, non-porous materials were prepared with varying weight % ratios of PCL:PLLA and the properties evaluated. The PCL-DA degree of polymerization (n; n = 25, 45) was also investigated. Findings included improved mechanical properties via an increase in 'n' (e.g. strength) and an increase in PLLA within the PCL:PLLA wt% ratio (e.g. modulus). Importantly, rates of degradation also increased with PLLA content within the PCL:PLLA wt% ratio.

These previous works concluded in realizing the potential for the PCL-PLLA semi-IPN SMP scaffolds in the regeneration of cranial bone defects. The rates of degradation observed were thought to be particularly interesting. However, the degradation analysis has previously been limited to accelerated (i.e. high pH solution conditions) testing.[12] Moreover, the effect of pore compression upon implant "fitting" on scaffold degradation has not been considered, despite the potential for the compression to impede the rate of water diffusion. Herein, a study to fully assess scaffold implant degradation has been conducted towards predicting *in vivo* implant behavior. PCL-PLLA semi-IPN scaffold implants were prepared via a solvent-casting particulate-leaching (SCPL) fabrication technique (**Figure 1**). Taking into account preparative implant considerations, the effects of compressed implant "fit" on degradation, in addition to the implications of the hydrophilic, polydopamine surface coating on degradation, were evaluated. Further, semi-IPN PCL:PLLA wt% ratios (100:0 [PCL-DA control], 90:10, 75:25, 60:40) and PCL-DA average degree of polymerization were varied (n = 25, 45). The PLLA average degree of polymerization (m) was maintained at m = 90. Degradation was evaluated under both accelerated conditions, to efficiently evaluate implant properties' effects on degradation, and under non-accelerated, real-time conditions, to closely simulate *in vivo* degradation.

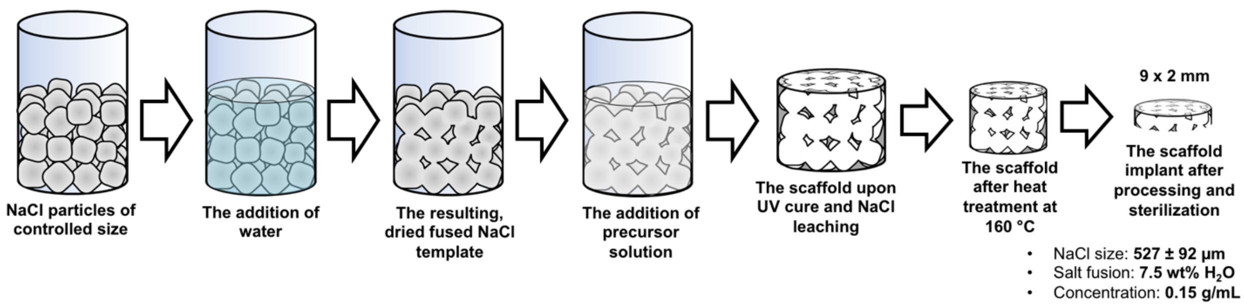

Figure 1. Schematic of SMP scaffold implant fabrication procedure.

SECTION II

MATERIALS AND METHODS

Materials

Polycaprolactone diol (PCL_{90}-diol; M_n ~ 10,000 g/mol), ε-caprolactone, L-lactide, stannous 2-ethylhexanoate, triethylamine (Et_3N), acryloyl chloride, 4-dimethylaminopyridine (DMAP), 2,2-dimethoxy-2-phenylacetophenone (DMP), 1-vinyl-2-pyrrolidinone (NVP), potassium carbonate (K_2CO_3), sodium hydroxide (NaOH), phosphate-buffered saline (PBS; 1X, pH = 7.4), ethylene glycol, and solvents were obtained from Sigma-Aldrich. Anhydrous magnesium sulfate ($MgSO_4$) was obtained from Fisher. Reagent CH_2Cl_2 and NMR-grade $CDCl_3$ were dried over 4 Å molecular sieves before use.

Polymer Synthesis

PCL_{2n}-diol (n = 25, 45) and $PLLA_{2m}$-diol (m = 90) were synthesized by the ring-opening polymerization of ε-caprolactone or l-lactide, respectively, with an ethylene glycol initiator and stannous 2-ethylhexanoate catalyst. The ethylene glycol initiator to monomer ratio controlled the degree of polymerization in both polymers (n, m). The resulting terminal hydroxyl groups of PCL_{2n}-diol were then replaced by photosensitive acrylate (OAc) groups by reacting with triethylamine and acryloyl chloride. The number average molecular weights (M_n) and degree of acrylation were determined by ^1H NMR.

Fabrication

In order to fabricate each specimen, SMP scaffolds were prepared using a previously reported solvent-casting particulate-leaching (SCPL) method.[13, 14] First, NaCl particles were sieved with openings of 425 µm resulting in particles with a diameter of 527 ± 92 µm. This was verified using ImageJ software and SEM images. 10 g of the collected NaCl particles were placed in a 20 mL glass vial and fused with 7.5 wt% DI water added in four increments and mechanically stirred after each addition. The mixture was centrifuged (3,220G, 15 minutes), air-dried for an hour, and dried *in vacuo* overnight at room temperature.

Macromer solutions (0.15 g mL^{-1} CH_2Cl_2, ~4 mL solution/ SMP scaffold) were prepared with varying PCL:PLLA wt% ratios (100:0, 90:10, 75:25, 60:40), along with 15 vol% photoinitiator solution (10 wt% DMP in NVP). The solutions were added to the salt template and centrifuged (1,260G, 10 minutes) prior to being exposed to UV light for 5 minutes and air-dried overnight. The SMP scaffolds were removed from the vials and submerged in water/ethanol (1:1 vol:vol) solution for 7 days with regular solution changes in order to leach out the NaCl particles (**Figure 1**).

After removal from solution and air-drying, the SMP scaffolds were annealed *in vacuo* at 160 °C for 10 minutes in order to induce the densification requisite for shape memory capabilities while also maintaining equivalent porosity between the PCL:PLLA ratios.[5] Each scaffold was then sectioned into three specimens (~1.75 mm thick) using a vibratome and punched to a 9 mm diameter. Finally, the implants were briefly treated at 85 °C to recover any deformation occurring during processing.

To investigate the effects on degradation of a compressed implant upon defect "fitting," select implants (75:25; PCL-DA, n = 45) underwent simulated "fitting" within a model defect. The fabricated, 9 mm implants were submerged in warm water (~60 °C, $T > T_{trans}$) and, via shape memory behavior, were subsequently "fitted" into an 8 mm model defect. Upon cooling ($T < T_{trans}$), the now-compressed implant was removed from the defect mold and dried *in vacuo* prior to evaluation of pore morphology and degradation rate under accelerated conditions.

Additionally, to study the effect of the a previously-studied, bioactive polydopamine coating on implant degradation, select implants (75:25; PCL-DA, n = 45) were surface-coated as previously reported.[6] Briefly, the fabricated implants were degassed using a syringe and suspended in a dopamine hydrochloride solution (2 mg mL^{-1} in 10 mM Tris buffer, pH = 8.5) at 150 rpm for 16 h. After, they were extensively rinsed with DI water and dried *in vacuo* prior to evaluation of degradation rate under accelerated conditions.

Characterization

Scanning Electron Microscopy

SEM imaging were used to compare the morphology of pores along the implant perimeter before and after simulated "fitting" for implants of the varying PCL:PLLA ratios. The implants were coated with Au-Pt, and then images were taken using a JEOL 6400 SEM with an accelerating voltage of 10 kV.

Accelerated Degradation

Implants (N = 3, per time point) were immersed in 10 mL of 0.1 M NaOH and maintained at 37°C at 60 rpm. At specified time-points, implants were removed and blotted dry followed by drying *in vacuo* overnight. The mass loss of each implant was determined and compared to the initial mass using Equation (1).

$$Mass\ Loss\ (\%) = \frac{m_{initial} - m_{final}}{m_{initial}} \times 100 \quad (1).$$

Mass loss of SMP implants of the varying PCL:PLLA wt% ratios (100:0, 90:10, 75:25, 60:40) and PCL molecular weight (n = 25, 45) was evaluated at 24, 72, 120, and 168 h. Mass loss of "fitted" implants (75:25; PCL-DA [n = 45]) and the corresponding "non-fitted" controls was evaluated at 120 h, and the mass loss of polydopamine-coated implants (75:25; PCL-DA [n = 45]) and the un-coated controls was evaluated at 24, 72, 120 and 168 h.

Neutral Degradation

Specimens (*N* = 3, per time point) comprised of the varying PCL:PLLA wt% ratios (100:0, 90:10, 75:25, 60:40) and PCL molecular weight (n = 25, 45) were immersed in 10 mL of phosphate-buffered saline (PBS, pH = 7.4) in a sealed 20 mL vial maintained at 37 °C at 60 rpm. At 1, 3, and 5 months, the respective samples were removed from the solution, blotted dry, and dried *in vacuo* overnight at room temperature. The mass of each specimen was determined and compared to the initial mass using Equation (1).

Water Uptake

Specimens (*N* = 3, per time point) comprised of the varying PCL:PLLA wt% ratios (100:0, 90:10, 75:25, 60:40) and PCL molecular weight (n = 25, 45) were each immersed in 10 mL of phosphate-buffered saline (PBS) in a sealed 20 mL vial maintained at 37 °C at 60 rpm. At 1, 3, and 5 months, the respective samples were removed from the solution and blotted dry. The mass of the implants before and after drying *in vacuo* was measured and used to calculate water update using Equation (2).

$$Water\ Uptake\ (\%) = \frac{m_{wet} - m_{dry}}{m_{dry}} \times\ 100 \qquad (2).$$

Statistical Analysis

Data was reported as the mean ± standard deviation. Values were compared using either two-way Anova or a Student’s t-test to determine p-values.

SECTION III

RESULTS AND DISCUSSIONS

Fabrication

Porous scaffolds were successfully fabricated using the SCPL method and 160 °C-annealing (**Figure 1**). Sectioning to a ~2 mm thickness and punching to a diameter of 9 mm resulted in a 9 x 2 mm scaffold implant. Previous work confirmed the PCL:PLLA wt% ratios via TGA and maintained scaffold properties (e.g. pore morphology) upon processing.[5]

Accelerated Degradation

In previous work,[5] degradation trends via mass loss under accelerated conditions (1 M NaOH, 37 °C) were studied using PCL-PLLA semi-IPN scaffold cylinders (5 x 12 mm). Although the general trends of more rapid degradation with increasing PLLA content within the semi-IPN PCL:PLLA wt% ratio, degradation occurred quickly for the 75:25 and 60:40 (PCL:PLLA wt%; PCL-DA [n = 45]) compositions, degrading completely by 72 h. In this work, scaffolds processed to implantation-ready discs (9 x 2 mm) were studied to better evaluate degradation. Additionally, the degradation solution concentration utilized for the accelerated hydrolysis testing (0.1 M NaOH, 37 °C, 60 rpm) was reduced in order to observe a fuller degradation profile. Additionally, the accelerated conditions (0.1 M NaOH, 37 °C, 60 rpm) were used to determine the effects of compressed implant "fit," as well as an applied polydopamine surface coating, on degradation. Both preparatory factors have the potential to affect degradation *in vivo* and, thus, were considered *in vitro*.

Since the rate of degradation depends on amount of surface area in contact with the medium, the compressed state of the implant after "fitting" within a defect could limit diffusion into the implant. Thus, the morphology and degradation behavior of a compressed, “fitted” 75:25 (PCL:PLLA wt%; PCL-DA [n = 45]) implant was compared to non-compressed implants. While the pore morphology along the compressed, “fitted” implant edge revealed signs of pore compression for all implant compositions (**Figure 2a**), hydrolytic degradation (75: 25 PCL:PLLA wt%; PCL-DA [n = 45]) via accelerated mass loss was not significantly affected at 120 h (**Figure 2b**). This could be attributed to the non-compressed state of the pores on the faces of the implant, which also possess a greater surface area than the “fitted” edges. As a result, *in vitro* degradation studies of initial, non-compressed implants are expected to be highly representative of analogs ultimately “fitted” within a cranial bone defect.

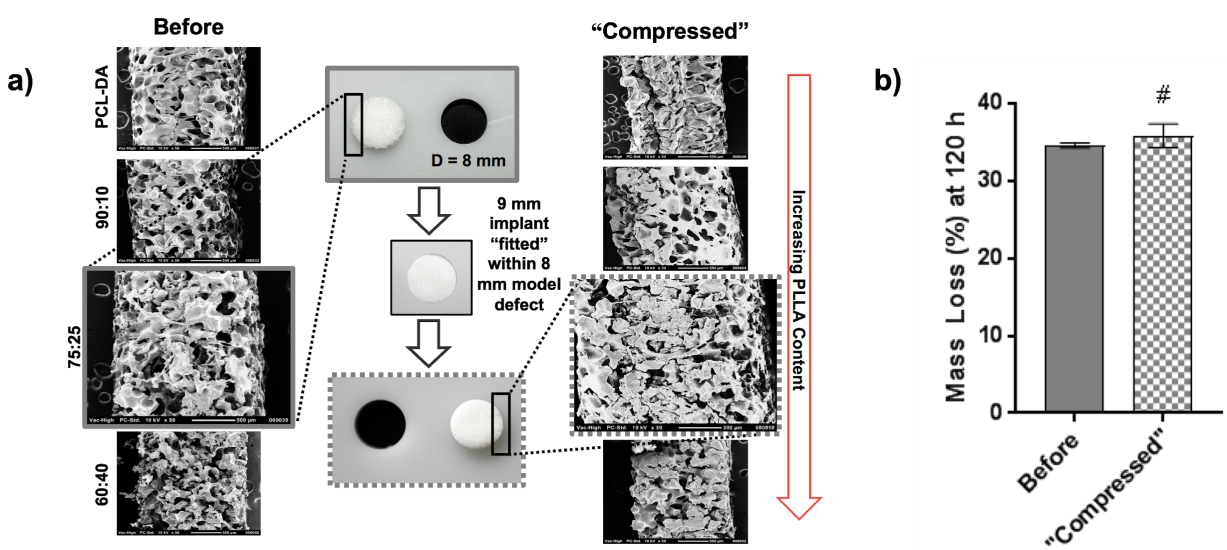

Figure 2. a) Photo series depicting a simulated implant “fitting” process within a model defect and SEM images of the circumferential implant edge before and after the “fitting.” Morphologically, the perimeter edge of the implants becomes slightly compressed after being “fitting” via shape memory. b) Mass loss under accelerated conditions (0.1 M NaOH, 37 °C, 60 rpm) for 75:25 (PCL:PLLA wt%) scaffold implants (PCL-DA [n = 45]) at 120 h ($^{\#}$ $p > 0.05$ vs corresponding non-compressed control).

In order to facilitate osteoinduction, a bioactive polydopamine coating can be applied to the surface of the implants. Previous work showed increased mineralization upon exposure to simulated body fluid and an osteogenic cellular response,[6] indicating potentially improved healing *in vivo*. In this study, implants (75:25 PCL:PLLA wt%; PCL-DA [n = 45]) were coated with polydopamine and subjected to accelerated degradation conditions (0.1 M NaOH, 37 °C, 60 rpm). The degradation rates via mass loss were then compared to an analogous, uncoated scaffold implant (**Figure 3**). The resulting degradation rate proved to be slightly increased for coated implants. This finding was expected due to dopamine's polar amine and hydroxyl functional groups. The coating also increases hydrophilicity of the surface and, consequently, hydrolysis. The observed tunability of degradation rate via scaffold coating could be favorable.

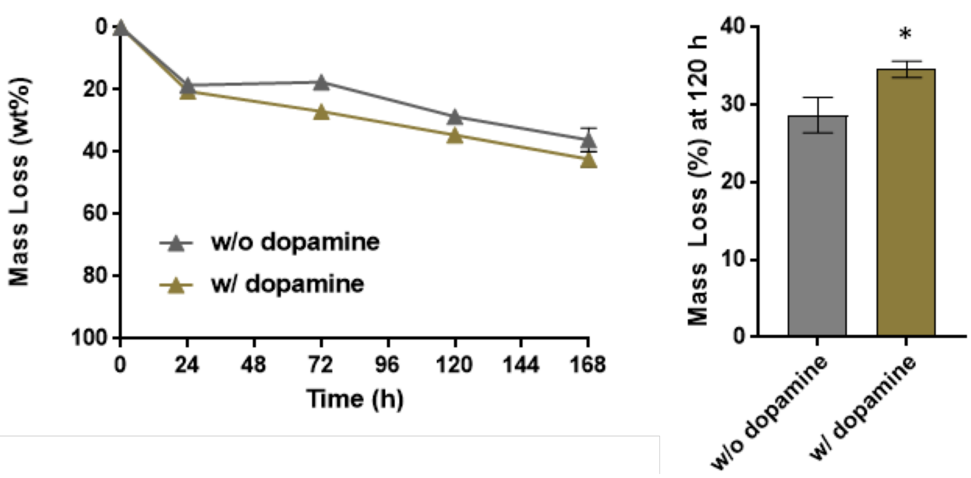

Figure 3. Mass loss under accelerated conditions (0.1 M NaOH, 37 °C, 60 rpm) for 75:25 (PCL:PLLA wt%) scaffold implants (PCL-DA [n = 45]) either without or with an applied polydopamine coating. Significance was observed at 120 h (*$p < 0.05$ vs corresponding uncoated control).

In addition to examining the effects of preparative implant considerations, the effect of the PCL:PLLA wt% ratio and PCL-DA 'n' on implant degradation were also evaluated under accelerated conditions (0.1 M NaOH, 37 °C, 60 rpm) (**Figure 4a**, **Figure 4b**). As hypothesized, implants with greater PLLA content degraded more quickly. After 168 h, the PCL-DA control (n = 25, 45) displayed a mass loss of only ~0.5% and ~2% respectively, while the 60:40 (PCL:PLLA wt%; PCL-DA [n = 25, 45]) compositions lost ~63% and ~79% mass. The observed differences between implants prepared with either PCL-DA (n = 25) or PCL-DA (n = 45) has been attributed to the increased number of hydrolytically-labile ester bonds in PCL-DA (n = 25) scaffolds.[15] Additionally, the implantable discs displayed visual fragmentation and decreased mechanical integrity at later stages of degradation (**Figure 4c**). The observation could indicate a bulk erosion mechanism.[16]

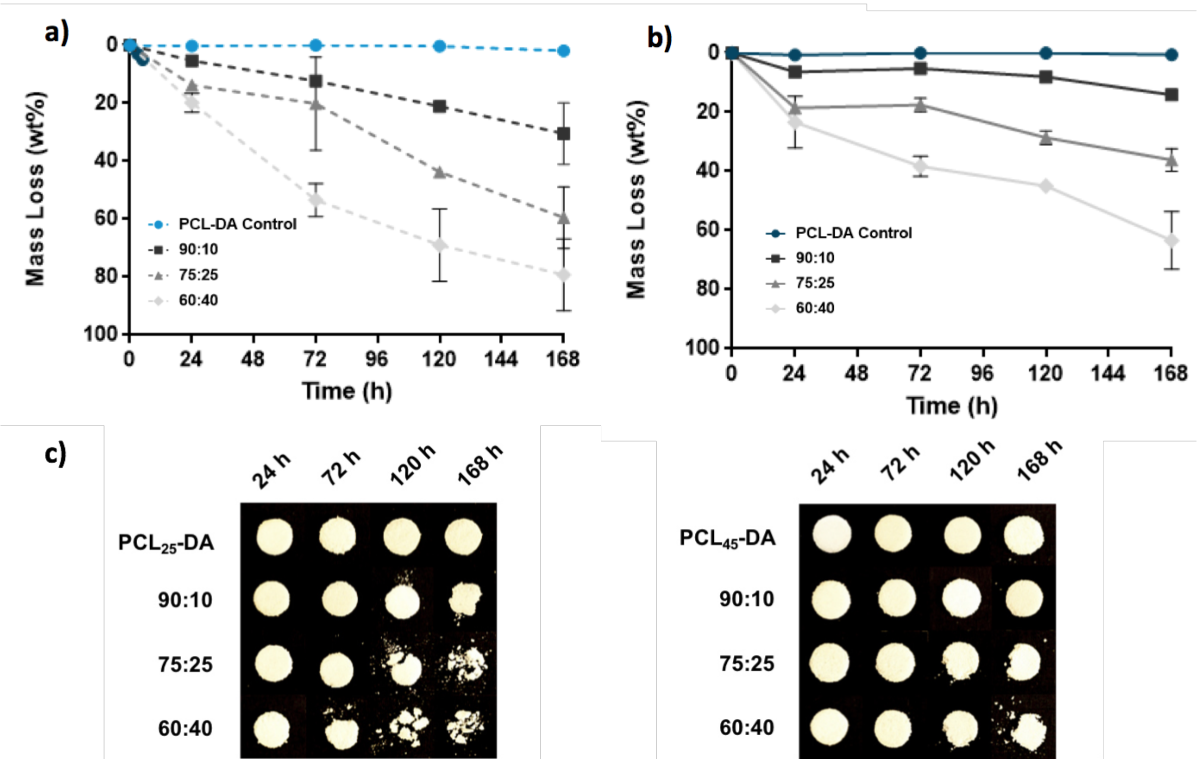

Figure 4. Mass loss under accelerated conditions (0.1 M NaOH, 37 °C, 60 rpm) for PCL-PLLA scaffold implants prepared with either a) PCL-DA (n = 25) or b) PCL-DA (n = 45). c) Images of scaffold implants at different time points throughout degradation.

Non-accelerated Degradation

In addition to accelerated testing, the PCL-DA control and PCL-PLLA semi-IPN scaffold implants were subjected to non-accelerated, real-time conditions (PBS [pH = 7.4], 37 °C, 60 rpm) to further investigate the degradation. Mass loss of the implants was assessed at 1, 3, and 5 months (**Figure 5a**, **Figure 5b**). Water uptake as also considered. By 5 months of non-accelerated degradation, mass loss was observed to increase, generally corresponding with PLLA content with the PCL-PLLA semi-IPN scaffolds. In this study, there was no observable difference in implant appearance post-degradation (**Figure 5c**). Thus, the mechanism of erosion is unclear pending a greater extent of degradation in future studies.

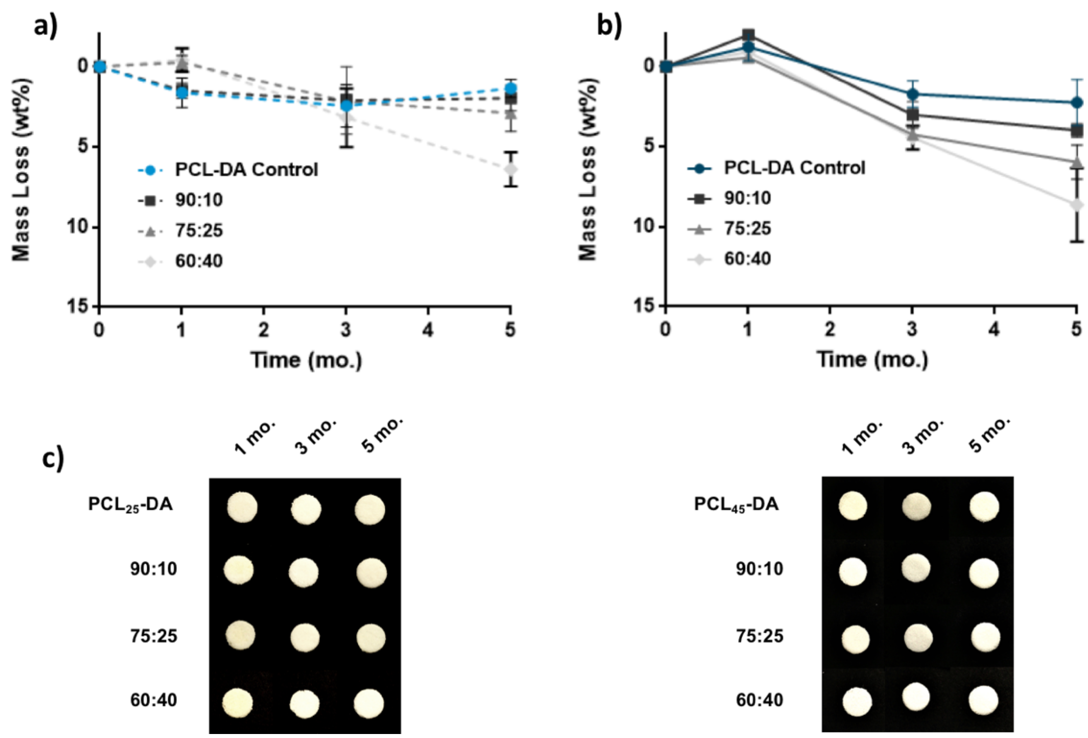

Figure 5. Mass loss under non-accelerated conditions (PBS [pH = 7.4], 37 °C, 60 rpm) for PCL-PLLA scaffold implants prepared with either a) PCL-DA (n = 25) or b) PCL-DA (n = 45). c) Images of scaffold implants at different time points throughout degradation.

At 5 months degradation, water uptake was quantified, and an increase in water uptake was observed in certain PCL-PLLA semi-IPN scaffold implants over the corresponding PCL-DA control implants (**Figure 6a**). The greater uptake implies an increase in water diffusion and absorption and could indirectly indicate greater hydrophilicity as well. The findings have been previously attributed to phase separation and a decrease in crystallinity.[5] Correspondingly, at 5 months, the PCL-DA controls prepared with PCL-DA (n = 25) and PCL-DA (n = 45) exhibited an average mass loss of ~1.4% and ~2.3%, respectively (**Figure 6b**). On the other hand, the 60:40 (PCL:PLLA wt%; PCL-DA [n = 25, 45]) scaffolds lost an average of ~6.4% and ~8.6% mass, respectively.

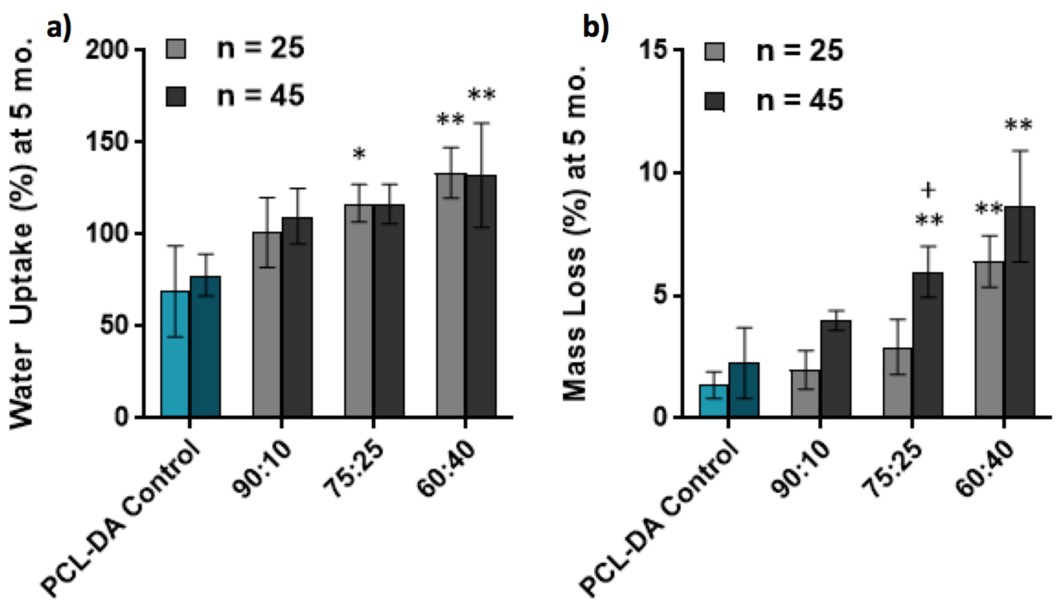

Figure 6. a) Water uptake under non-accelerated conditions (PBS [pH = 7.4], 37 °C, 60 rpm) for PCL-PLLA scaffold implants at 5 months (*p < 0.05, **p < 0.01 vs corresponding PCL-DA control implant). b) Corresponding mass loss under non-accelerated conditions (PBS [pH = 7.4], 37 °C, 60 rpm) for PCL-PLLA scaffold implants at 5 months (**p < 0.01 vs corresponding PCL-DA control implant; +p < 0.05 vs corresponding PCL-DA (n = 25) composition).

The amount of PLLA within the semi-IPN PCL:PLLA wt% ratio contributed to greater rates of mass loss and degradation under both accelerated and non-accelerated conditions. Under non-accelerated conditions, however, an increase in degradation was observed for 75:25 (PCL:PLLA wt% ratio) implants when prepared with PCL-DA (n = 45) over PCL-DA (n = 25) implants. While different from observations under accelerated conditions, the finding may be due to a reduced crosslink density within the PCL-DA (n = 45) scaffolds, despite no noticeable difference in measured water uptake. Further studies will discern if the observed differences can be attributed to differences in semi-IPN scaffold properties or if there is a limitation of water uptake analysis.

CONCLUSION

Porous, SMP scaffold implants comprised of semi-IPNs of cross-linked PCL-DA and thermoplastic PLLA have the potential to be “fitted” within irregular defects, promoting healing while also possessing favorable and tunable properties. In this work, scaffold implant degradation and tunability were further assessed. Accelerated degradation testing (0.1 M NaOH, 37 °C, 60 rpm) investigated the effect of preparative implant considerations on degradation. The designed process of “fitting” scaffold implants within cranial bone defects via shape memory behavior was found to have no significant effect on degradation rate. Additionally, a developed, bioactive polydopamine coating applied to the surfaces of the scaffold implants contributed to greater rates of degradation. Lastly, the porous implants exhibited greater extends of mass loss with increasing PLLA content within the semi-IPN PCL:PLLA wt% ratio.

Furthermore, non-accelerated testing (PBS [pH = 7.4], 37 °C, 60 rpm) served to best simulate and predict *in vivo* degradation behavior. Degradation initially closely paralleled the trends observed under accelerated degradation conditions, and water uptake was determined to be greater in semi-IPNs with greater PLLA content within the PCL:PLLA wt% ratio. Future work will study such degradation up to twelve months, providing additional conclusions.

REFERENCES

[1] W. Weng, S. Song, L. Cao, X. Chen, Y. Cai, H. Li, Q. Zhou, J. Zhang, J. Su*, J. Nanomater.* **2014**, 236.

[2] N.-S. Choi, C.-H. Kim, K. Y. Cho, J.-K. Park, *J. Appl. Polym. Sci.* **2002**, 86, 1892.

[3] M. Lebourg, J. S. Antón, J. L. G. Ribelles, *Eur. Polym. J.* **2008**, 44, 2207.

[4] L. Woodard, V. Page, K. Kmetz, and M. Grunlan. *Macromol. Rapid Commun.* **2016**, 37, 1972–1977.

[5] L. Woodard, K. Kmetz, A. Roth, V. Page, M. Grunlan. *Biomacromolecules.* **2017,** 18, 4075-4083.

[6] D. Zhang, O. George, K. Petersen, A. Jimenez-Vergara, M. Hahn, M. Grunlan, *Acta Biomater.* **2014**, 10, 4597–4605.

[7] A. Lendlein, S. Kelch. *Angew Chem. Int. Ed.* **2002**, 41, 2034–57.

[8] E. Neovius, T. Engstrand. *J. Plast. Reconstr. Aesthet. Surg.* **2010**, 63, 1615–23.

[9] M. A. Woodruff, D. W. Hutmacher. *Prog. Polym. Sci.* **2010**, 35, 1217–56.

[10] L. A. Gaona, J. L. Gómez Ribelles, J. E. Perilla, M. Lebourg, *Polym. Degrad. Stab.* **2012**, 97, 1621.

[11] A. Lendlein, A. M. Schmidt, M. Schroeter, R. Langer. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, 43, 1369.

[12] F. von Burkersroda, L. Schedl, A. Göpferich*, Biomaterials.* **2002**, 23, 4221.

[13] D. Zhang, M. L. Giese, S. L. Prukop, M. A. Grunlan. *J. Polym. Sci. Pol. Chem.* **2011**, 49, 754.

[14] C. A. Schoener, C. B. Weyand, R. Murthy, M. A. Grunlan. *J. Mater. Chem.* **2010**, 20, 1787.

[15] X.-J. Xu, J. C. Sy, V. Prasad Shastri. *Biomaterials* **2006**, 27, 3021.

[16] A. Göpferich. *Macromolecules.* **1997**, 30, 2598-2604.